Remarks

Claims 1-56 were initially pending in the subject application. In response to a restriction requirement, claims 1-16, 30, and 51-52 were elected for examination on August 16, 2000. By way of the amendment of this date, claims 57-58 have been added. Therefore, claims 1-16, 30, 51-52 , and 57-58 are now before the Examiner for consideration. The subject invention provides unique and advantageous *Chlamydia trachomatis* polynucleotides, vectors, transformed host cells, DNA chips, and kits containing the aforementioned polynucleotides. Certain of the claims have been amended for the purpose of expediting the patent application process in a manner consistent with the Patent and Trademark Office Patent Business Goals (PBG), 65 Fed. Reg. 54603 (September 8, 2000), advancing prosecution, and facilitating the business interests of Applicants. Support for these new claims and the amendments to the pending claims can be found throughout the subject specification, including, for example, the originally presented claims or at pages 8-9. Favorable consideration of the claims now presented, in view of the remarks and amendments set forth herein, is earnestly solicited.

The Office Action of April 17, 2001 has objected to the title of the application, asserting that the title is not descriptive of the subject matter to which the claims are directed. Applicants have amended the title and believe this issue to be moot.

Claims 1-16, 30, and 51-52 have been rejected under 35 U.S.C. §101 as lacking patentable utility due to its not being supported by either a specific and/or substantial utility or a well-established utility. The Office Action alleges that the specification fails to connect the claimed invention to any particular or specific utility and that no substantial utility has been established for the claimed subject matter. Applicants respectfully traverse.

Applicants submit that the claimed subject matter has patentable utility (*i.e.*, "specific", "substantial", "credible", and "well-established" utility) and meets the definitions set forth in the Office Action of April 17, 2001 at pages 2-3. Particularly, the claimed nucleic acids are useful as hybridization probes for the detection of *C. trachomatis*. As evidence of such usefulness, it is respectfully submitted that the art generally recognizes and practices the detection of *Chlamydia trachomatis* in biological samples by utilizing nucleic acid detection methodologies. These detection methodologies include PCR and/or hybridization assays (see attached ViroMed Laboratories and GEN-PROBE publications and references cited therein). As is evident from these publications, one

skilled in the art would recognize a "well-established" utility for the disclosed polynucleotide sequences as reagents for the detection of *Chlamydia trachomatis* infections in humans in PCR or hybridization assays and such a use would have been readily apparent to one skilled in the art alone, or in view of the instant disclosure, and/or in view of the knowledge of the skilled artisan.

Furthermore, the disclosure of the instant specification, alone or in combination with the publications provided in this response, support, "specific", "substantial", and "credible" utilities for the instant subject matter. As the Examiner will recognize, the claimed polynucleotide sequences are useful as hybridization probes or PCR probes for the detection of *C. trachomatis* in biological samples by virtue of the sequences having been obtained from this organism. Furthermore, a "substantial" utility can be assigned to the claimed polynucleotides because nucleic acid assay systems are commercially available or are provided by "real-world" vendors that utilize *C. trachomatis* nucleic acid probes within their assay systems. Thus, the claimed polynucleotide sequences would be useful in such assay systems. Finally, the asserted utility of the invention in nucleic acid detection assays (either PCR-based systems: specification at page 49, lines 25-29; or hybridization assays: page 51, lines 3-31) is credible as assessed from the perspective of one of ordinary skill in the art. Thus, it is respectfully submitted that the claimed polynucleotide sequences possess a patentable utility and withdrawal of the rejection is respectfully requested.

Claims 1-16, 30, and 51-52 have been rejected under 35 U.S.C. § 112, first paragraph, because one skilled in the art would not know how to use the claimed subject matter because the claimed invention is not supported by a specific, substantial, and credible utility, or alternatively, a well-established utility. As indicated *supra*, it is respectfully submitted that the claimed polynucleotide sequences are useful in nucleic acid based assays for the detection of *C. trachomatis* in biological samples. As such, it is respectfully submitted that one skilled in the art would know how to use the claimed subject matter. Reconsideration and withdrawal of the rejection is respectfully requested.

Claims 1-16, 30, and 51-52 have been rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the invention was filed, had possession of the claimed invention. Applicants respectfully traverse.

The subject invention is directed to novel and non-obvious open reading frames identified and isolated from *Chlamydia trachomatis*. It is respectfully submitted that one skilled in the art can readily envisage nucleic acid sequences containing the recited ORFs because these nucleic acids can be readily inserted into known replication vectors. Applicants also respectfully submit that one skilled in the art would be able to readily envisage nucleic acid sequences having 80% or 99.9% homology to the claimed ORFs. Accordingly, withdrawal of the rejection is respectfully requested.

The subject invention also provides polynucleotides that hybridize with the open reading frames of the invention, provided that the hybridizing polynucleotides have at least 80% or 99.9% homology to the complementary polynucleotide sequences of the claimed ORFs. Given that the full length open reading frames of the invention are provided in the disclosure, Applicants respectfully submit that one skilled in the art would be able to envision the sequences having the required degree of homology to the complementary open reading frames. Accordingly, withdrawal of the rejection is respectfully requested.

Claims 1-7, 51, and 52 have been rejected under 35 U.S.C. §§ 102(a) or 102(b) as being anticipated by a variety of Genbank accession numbers. Applicants respectfully submit that the prior art applied in the Office Action does not anticipate the claimed sequences and fails to teach these same polynucleotide sequences. Furthermore, it is respectfully submitted that the prior art polynucleotide sequences fail to demonstrate the requisite degree of homology necessary to sustain an anticipation rejection for hybridizing polynucleotide sequences. Reconsideration and withdrawal of the rejections is respectfully requested.

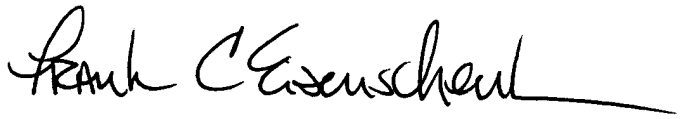
Claims 1-7, 9-11, 13, 15, 51, and 52 were rejected under 35 U.S.C. § 103(a) as being unpatentable over the Genbank sequences. The Office Action states that it would have been obvious to one of skill in the art to link the Genbank sequences to regulatory elements, prepare transformed host cells containing the sequences in operatively linked vectors, and make complements of the sequences. It is well settled law that all the claim limitations must be taught or suggested by the prior art to establish *prima facie* obviousness of a claimed invention. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). Applicants respectfully submit that the Genbank sequences do not teach the claimed polynucleotide sequences nor do the Genbank sequences teach all the limitations found in the claims. Withdrawal of this obviousness rejection is respectfully requested.

Claim 30 was rejected under 35 U.S.C. § 103(a) as being unpatentable over the Genbank sequences in view of Southern *et al.* (U.S. Patent No. 5,436,327). The Office Action states that the Genbank sequences describe nucleic acid sequences within the scope of the instant claims and Southern *et al.* teach the preparation and use of DNA chips. Applicants respectfully submit that the Genbank sequences do not constitute prior art applicable to the claims, fail to teach each and every limitation of the claims, and, therefore, the rejection fails to raise a *prima facie* case of obviousness for claim 30. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

In view of the foregoing remarks and the amendments to the claims, Applicants believe that the pending claims are now in condition for allowance, and such action is respectfully requested. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants also invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephone interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



Frank C. Eisenschenk, Ph.D.
Patent Attorney
Registration No. 45,332
Phone: 352-375-8100
Fax No.: 352-372-5800
Address: Saliwanchik Lloyd & Saliwanchik
A Professional Association
2421 NW 41st Street, Suite A-1
Gainesville, FL 32606-6669

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Attachments:
- Marked-Up Version of Amended Title
- Marked-Up Version of Amended Claims
- "C. Trachomatis & N. Gonorrhoeae PCR", ViroMed Laboratories, October 2000
- "Chlamydia Trachomatis and Neisseria Gonorrhoeae", Gen-Probe Inc., 103906 Rev. K/April 17, 2001

Marked-up Title

~~CHLAMYDIA TRACHOMATIS GENOMIC SEQUENCE AND POLYPEPTIDES, FRAGMENTS THEREOF AND USES THEREOF, IN PARTICULAR FOR THE DIAGNOSIS, PREVENTION AND TREATMENT OF INFECTION~~

CHLAMYDIA TRACHOMATIS NUCLEIC ACIDS AND USES THEREOF

Marked-up Claims

Claim 1 (Amended):

An isolated polynucleotide ~~having a nucleotide~~ sequence ~~of a~~ obtained from *Chlamydia trachomatis* ~~genome,~~ comprising the polynucleotide sequence of SEQ ID NO. 1083, SEQ ID NO. 1089, SEQ ID NO. 1091, SEQ ID NO. 1095, SEQ ID NO. 1096, SEQ ID NO. 1105, SEQ ID NO. 1117, SEQ ID NO. 1140, SEQ ID NO. 1159, or SEQ ID NO. 1167; or

an isolated polynucleotide sequence having at least 80% homology to SEQ ID NO. 1083, SEQ ID NO. 1091, SEQ ID NO. 1095, SEQ ID NO. 1096, SEQ ID NO. 1105, SEQ ID NO. 1117, SEQ ID NO. 1140, SEQ ID NO. 1159, or SEQ ID NO. 1167; or

an isolated polynucleotide sequence having at least 99.9% homology to SEQ ID NO. 1089, SEQ ID NO. 1083, SEQ ID NO. 1091, SEQ ID NO. 1095, SEQ ID NO. 1096, SEQ ID NO. 1105, SEQ ID NO. 1117, SEQ ID NO. 1140, SEQ ID NO. 1159, or SEQ ID NO. 1167.

~~(a) the nucleotide sequence of SEQ ID No. 1;~~

~~(b) the nucleotide sequence contained within the *Chlamydia trachomatis* genomic DNA in ECACC Deposit No. 98112618;~~

~~(c) the nucleotide sequence contained in a clone insert in ECACC Deposit No. 98112617;~~

~~(d) a nucleotide sequence exhibiting at least 99.9% identity with the sequence of SEQ ID No. 1; or~~

~~(e) a nucleotide sequence exhibiting at least 80% homology to SEQ ID No. 1.~~

Claim 2 (Amended):

An isolated polynucleotide ~~which hybridizes to SEQ ID No. 1 or to the *Chlamydia trachomatis* genomic DNA contained in ECACC Deposit No. 98112618 or to a clone insert in ECACC Deposit No. 98112617 under conditions of high stringency.~~ that hybridizes to a *Chlamydia trachomatis* polynucleotide sequence comprising the sequence of SEQ ID NO. 1083, SEQ ID NO. 1089, SEQ ID NO. 1091, SEQ ID NO. 1095, SEQ ID NO. 1096, SEQ ID NO. 1105, SEQ ID NO. 1117, SEQ ID NO. 1140, SEQ ID NO. 1159, or SEQ ID NO. 1167, under conditions of high stringency, wherein said hybridizing polynucleotide sequence has at least 80% homology to the

complementary sequence of SEQ ID NO. 1083, SEQ ID NO. 1091, SEQ ID NO. 1095, SEQ ID NO. 1096, SEQ ID NO. 1105, SEQ ID NO. 1117, SEQ ID NO. 1140, SEQ ID NO. 1159, or SEQ ID NO. 1167, or at least 99.9% homology to the complementary sequence of SEQ ID NO. 1089.

Claim 3 (Amended):

An isolated polynucleotide ~~which hybridizes to SEQ ID No. 1 or to the *Chlamydia trachomatis* genomic DNA contained in ECACC Deposit No. 98112618 under conditions of intermediate stringency.~~ that hybridizes to a *Chlamydia trachomatis* polynucleotide sequence comprising the sequence of SEQ ID NO. 1083, SEQ ID NO. 1089, SEQ ID NO. 1091, SEQ ID NO. 1095, SEQ ID NO. 1096, SEQ ID NO. 1105, SEQ ID NO. 1117, SEQ ID NO. 1140, SEQ ID NO. 1159, or SEQ ID NO. 1167 under conditions of intermediate stringency, wherein said hybridizing polynucleotide sequence has at least 80% homology to the complementary sequence of SEQ ID NO. 1083, SEQ ID NO. 1091, SEQ ID NO. 1095, SEQ ID NO. 1096, SEQ ID NO. 1105, SEQ ID NO. 1117, SEQ ID NO. 1140, SEQ ID NO. 1159, or SEQ ID NO. 1167or at least 99.9% homology to the complementary sequence of SEQ ID NO. 1089.

Claim 8 (Amended):

A polynucleotide encoding a fusion protein, comprising one of ORF2 to ORF 1197 of Claim ~~4, 5 or 6~~ 1, 2 or 3 ligated in frame to a polynucleotide encoding a heterologous polypeptide.

CHLAMYDIA TRACHOMATIS & NEISSERIA GONORRHOEAE PCR DETECTION PANEL

ViroMed Laboratories performs the COBAS AMPLICOR CT/NG PCR test for Chlamydia trachomatis and Neisseria gonorrhoeae nucleic acid detection. Nucleic acid amplification is more sensitive and specific than the direct probe test, and using PCR (Polymerase Chain Reaction) for both C. trachomatis and N. gonorrhoeae means that a single sample can now be submitted for both tests. The tests are available separately or as a panel.

TEST METHODOLOGY

Polymerase chain reaction (PCR) technology is used to amplify target DNA, and product is detected using a hybridization capture in an automated analyzer (COBAS AMPLICOR).

BACKGROUND

The two most commonly reported sexually transmitted diseases in 1996 were C. trachomatis and N. gonorrhoeae and it was anticipated that new cases would extend into the millions annually thereafter. Both infections can be asymptomatic in either men or women (50-80%) and the sequeale of undiagnosed infections in women is usually the most severe (e.g., pelvic inflammatory disease, infertility and neonatal disease). Therefore, a need for the most sensitive and specific test is clearly warranted. Currently amplification assays, such as the polymerase chain reaction, satisfy this requirement over conventional testing (e.g., culture or DFA).

There have been numerous publications on the AMPLICOR Chlamydia trachomatis assay exhibiting high sensitivity (95-99%) and specificity (99-100%). [See references on reverse side.] Most recently, a large multicenter evaluation (1,253 female, 251 male) of the COBAS AMPLICOR CT/NG test for C. trachomatis by Vincelette, et. al. has shown the following results:

	FEMALE SWAB	FEMALE URINE	MALE SWAB	MALE URINE
SENSITIVITY	96.5	95.1	100.0	94.4
SPECIFICITY	99.4	99.8	98.5	100.0

An internal control provided in the COBAS AMPLICOR test also showed an initial low 2.9% inhibition rate and after retesting a second aliquot from the original specimen the rate dropped to <1%. This automated PCR test exhibits high sensitivity and specificity, low test inhibition and can use a noninvasive specimen (urine) to generate same day test results.

Testing for N. gonorrhoeae using the COBAS AMPLICOR CT/NG system in a study by Higgins et. al with a gonorrhoeae prevalence of 9.49% showed no significant difference in sensitivity or specificity when compared to culture. Multiplexing is also a distinct advantage in testing for both STD agents.

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C. trachomatis & N. gonorrhoeae PCR

ORDERING INFORMATION

	C. trachomatis and *N. gonorrhoeae* DNA PCR Detection Panel	*Chlamydia trachomatis* DNA PCR	*Neisseria gonorrhoeae* DNA PCR
Test Codes	**60202**	**60034**	**60203**
Method	Polymerase Chain Reaction		
Specimen Requirements	Endocervical swab (**female**), urethral swab (**male**) in VTM (ViroMed Viral Transport Media) at 2-8°C **OR** Urine (10 ml – 50 ml first catch) ***(Urines are stable for 24hrs at 18-25°C and should be stored at 2-8°C after 1-7 days.)***		
Shipping Requirements	Room or refrigerated temperature overnight		
Days Set Up	Monday – Friday		
Results Available	Within 24 hours (Mon – Fri)		
CPT Code	87491 (***C. trachomatis*** amplified probe technique) 87591 (***N. gonorrhoeae*** amplified probe technique)		

The PCR (Polymerase Chain Reaction) test is performed pursuant to an agreement with Roche Molecular Systems, Inc.

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GEN-PROBE®

PACE® 2C

CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE

For screening of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* in Endocervical and Male Urethral Specimens

INTENDED USE

The GEN-PROBE® PACE® 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE is a DNA probe test that utilizes nucleic acid hybridization technology to screen for the presence of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* from endocervical and male urethral swab specimens collected with the GEN-PROBE PACE Specimen Collection Kits. Follow-up testing in individual *C. trachomatis* and *N. gonorrhoeae* assays is needed to identify the organism(s) present in PACE 2C-positive specimens.

SUMMARY AND EXPLANATION OF THE TEST

Chlamydiae are nonmotile, Gram-negative, obligate intracellular bacteria that rely on the ATP produced by the host cell for replication. Chlamydial infections have been known for many years. However, appreciation of the pathogenicity of *Chlamydia trachomatis* has increased over time (24, 26). *C. trachomatis* is responsible for approximately 50-80% of the cases of nongonococcal urethritis (10, 11).

The *Chlamydia trachomatis* species is comprised of fifteen serovars that are responsible for the following diseases in humans: trachoma, inclusion conjunctivitis, lymphogranuloma venereum, and other sexually transmitted diseases. The serovars D through K are the major cause of genital chlamydial infections in men and women (27). Clinical signs produced by *Chlamydia trachomatis* in humans include nongonococcal urethritis, epididymitis, proctitis, cervicitis, and acute salpingitis (10, 25, 28). In addition to the sexual transmission of chlamydial infections, newborn children are significantly at risk for inclusion conjunctivitis and chlamydial pneumonia from infected mothers (1, 8, 29).

Several methods have been used to detect the presence of *Chlamydia trachomatis* in infected tissue. These include direct Giemsa staining of infected tissue and evaluation by light microscopy and cell culture of specimens followed by visualization of inclusion bodies by iodine, fluorescein, or conjugated antibody staining (6, 20, 27, 32). More recently, rapid methods have been developed using antigen detection and nucleic acid hybridization (14, 15).

Gonorrhea is a very commonly reported bacterial infection in the United States, with nearly 392,200 cases reported in 1993 (5). This sexually transmitted disease usually results in anterior urethritis accompanied by a purulent exudate in men. In women, the disease is most often found in the cervix, but the vagina and uterus also may be infected. While severe complications and sterility can occur in untreated individuals, asymptomatic infections are frequently diagnosed. Gonorrhea infections also may be diagnosed from other mucous membranes including the conjunctiva, anus, and oropharynx (18).

Neisseria gonorrhoeae is the causative agent of gonorrhea. *N. gonorrhoeae* is a Gram-negative, oxidase-positive diplococcus that has stringent growth requirements (9, 13, 30). Presumptive diagnosis of gonorrhea is based on recovery of the organism from culture, morphological examination using gram stain, and determination of the presence of cytochrome oxidase (9, 13). Confirmatory procedures for the definitive diagnosis of gonorrhea infections include fluorescent antibody staining, carbohydrate degradation, agglutination, sugar fermentation, and nucleic acid hybridization tests (7, 12, 19, 23, 31, 17). More recently, nucleic acid hybridization has been used to diagnose gonorrhea infections directly from patient samples (22).

The GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE uses nucleic acid hybridization technology (16) to detect *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* directly from endocervical and male urethral swab specimens. The assay does not distinguish between the two organisms, but indicates if one or both are present in a specimen.

PRINCIPLES OF THE PROCEDURE

Nucleic acid hybridization tests are based on the ability of complementary nucleic acid strands to specifically align and associate to form stable double-stranded complexes (16). The GEN-PROBE PACE 2C System uses single-stranded DNA probes with chemiluminescent labels that are complementary to the ribosomal RNA of the target organisms. After the ribosomal RNA is released from the organisms, the labeled DNA probes combine with the ribosomal RNA of the target organisms to form stable DNA:RNA hybrids. The labeled DNA:RNA hybrids are separated from the non-hybridized probes and are measured in a GEN-PROBE LEADER luminometer. The test results are calculated as the difference between the response of the specimen and the mean response of the Negative Reference.

REAGENTS

The GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE

PACE 2 Hybridization Buffer. Buffered solution containing <20% detergent.

PACE 2C *Chlamydia trachomatis/Neisseria gonorrhoeae* Probe Reagent. Lyophilized labeled non-infectious DNA probe (<500 ng/vial).

PACE 2 Selection Reagent. Buffered solution containing <8% detergent.

PACE 2 STD Separation Reagent. Solid phase (1.25 mg/mL) in a solution containing 0.02% sodium azide as a preservative.

PACE 2 STD Negative Reference. Non-infectious nucleic acid in a buffered solution containing <5% detergent.

PACE 2 *Chlamydia trachomatis* Positive Control. Non-infectious *C. trachomatis* nucleic acid in a buffered solution containing <5% detergent.

PACE 2 *Neisseria gonorrhoeae* Positive Control. Non-infectious *N. gonorrhoeae* nucleic acid in a buffered solution containing <5% detergent.

PACE 2 STD Wash Solution. Buffered solution containing <2% detergent.

Sealing Cards. One package.

GEN-PROBE DETECTION REAGENT KIT (Provided Separately)

Detection Reagent I. 0.1% Hydrogen peroxide in 0.001N nitric acid.

Detection Reagent II. 1N Sodium hydroxide.

WARNINGS AND PRECAUTIONS

A. For *in vitro* diagnostic use.

B. This test system has been evaluated using endocervical and male urethral swab specimens only. Performance with other specimen types has not been assessed.

C. Separation Reagent MUST NOT freeze. The performance of the assay will be affected by use of improperly stored Separation Reagent. If the reagent has been frozen, the particles in the suspension may clump, resulting in a granular appearance that will not evenly disperse after thorough mixing. Visible clumps of Separation Reagent may adhere to the walls of the container. If this occurs, contact GEN-PROBE Technical Service.

D. Clean laboratory ware must be used to prepare reagents. Disposable polystyrene containers are strongly recommended.

E. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

F. Specimens may be infectious. Use universal precautions (3). Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this type of diagnostic procedure.

1. Thoroughly clean and disinfect all work surfaces.
2. Autoclave any contaminated equipment or materials that have come in contact with the samples before discarding.

G. Separation Reagent contains sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. Upon disposal of this reagent, always dilute the material with a large volume of water to prevent azide buildup in the plumbing.

H. Avoid contact of Detection Reagents I and II with skin, eyes and mucous membranes. **WARNING: IRRITANTS, CORROSIVES.** Wash with water if these reagents come into contact with skin or eyes. If spills of these reagents occur, dilute with water before wiping dry.

I. **DO NOT** interchange, mix or combine reagents from kits with different lot numbers except for STD wash solution.

STORAGE AND HANDLING REQUIREMENTS

PACE 2C *Chlamydia trachomatis/Neisseria gonorrhoeae* Probe Reagent and PACE 2 Separation Reagent must be stored at 2° to 8°C.

The PACE 2C *Chlamydia trachomatis/Neisseria gonorrhoeae* Probe Reagent is stable for 3 weeks after reconstitution when stored at 2° to 8°C.

The prepared Separation Suspension is stable for 6 hours after preparation when stored at 20° to 25°C.

Other reagents contained in the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE are to be stored at 2° to 25°C and are stable until the date stamped on the container.

DO NOT FREEZE THE REAGENTS CONTAINED IN THIS KIT.

SAMPLE COLLECTION AND STORAGE

The GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE is designed to screen for the presence of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* in endocervical and male urethral specimens collected using the GEN-PROBE PACE SPECIMEN COLLECTION KITS.

Only swabs contained in the PACE SPECIMEN COLLECTION KITS can be used to collect patient specimens. The swabs collected from patients MUST BE transported to the laboratory in the GEN-PROBE transport medium.

A. Collect swab samples as follows:

1. Cervical swab specimens
   a. Remove excess mucus from the cervical os and surrounding mucosa using one of the swabs provided in the cervical collection kit and discard the swab.
   b. Insert the second swab from the collection kit into the endocervical canal.
   c. Rotate the swab for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
   d. Withdraw the swab carefully; avoid any contact with the vaginal mucosa.
   e. **Fully insert** the swab into the GEN-PROBE transport tube.
   f. Break the swab shaft at the scoreline to fit the tube; use care to avoid splashing of contents. **Cap the tube tightly.**
2. Urethral swab specimens
   a. Patient should not have urinated for at least 1 hour prior to sample collection.
   b. Insert the swab from the urethral/conjunctival collection kit 2 to 4 cm into the urethra using a rotating motion to facilitate insertion.
   c. Once inserted, rotate the swab gently using sufficient pressure to ensure the swab comes into contact with all urethral surfaces. Allow the swab to remain inserted for 2 to 3 seconds.
   d. Withdraw the swab.
   e. **Fully insert** the swab into the GEN-PROBE transport tube.
   f. Break the swab shaft at the scoreline to fit the tube; use care to avoid splashing of contents. **Cap the tube tightly.**

B. Transport the tubes to the laboratory at 2° to 25°C and store at 2° to 25°C until tested. Samples should be assayed with the GEN-PROBE PACE 2C System within 7 days. If longer storage is necessary, process the specimen as described in SAMPLE PREPARATION and freeze at -20° to -70°C.

C. During routine analysis, bloody specimens have not proven to interfere with assay performance. However, grossly bloody specimens (greater than 80 µL whole blood in 1 mL transport medium) may interfere with performance.

D. Specimens which require shipping should be transported to the laboratory in compliance with regulations covering transportation of etiological agents. Store and test as described (see B above).

MATERIALS PROVIDED

The GEN-PROBE® PACE® 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE

	Cat. No. 3905 100 Tests	Cat. No. 3905B 1000 Tests
PACE 2 Hybridization Buffer	2 x 6 mL	20 x 6 mL
PACE 2C Chlamydia trachomatis/ Neisseria gonorrhoeae Probe Reagent	2 x 6 mL (when reconstituted)	20 x 6 mL (when reconstituted)
PACE 2 Selection Reagent	1 x 100 mL	10 x 100 mL
PACE 2 STD Separation Reagent	1 x 9 mL	10 x 9 mL
PACE 2 STD Negative Reference	1 x 7 mL	10 x 7 mL
PACE 2 Chlamydia trachomatis Positive Control	1 x 3 mL	10 x 3 mL
PACE 2 Neisseria gonorrhoeae Positive Control	1 x 3 mL	10 x 3 mL
PACE 2 STD Wash Solution	3 x 200 mL	2 x 3800 mL
Sealing Cards	1 package	10 packages
GEN-PROBE Disposable Polystyrene Reaction Tubes (12 x 75 mm)	120 tubes/box	10 x 120 tubes/box

MATERIALS REQUIRED BUT NOT PROVIDED

GEN-PROBE® PACE® Specimen Collection Kits
GEN-PROBE® Detection Reagent Kit
Magnetic Separation Unit
Vortex Mixer
Covered water bath (60° ± 1°C)
Micropipettes (100 µL)
Pipettes capable of delivering 1-25 mL
Absorbent paper

MATERIALS AVAILABLE FROM GEN-PROBE

GEN-PROBE® PACE® Specimen Collection Kits
Urethral/Conjunctival: (Cat. No. 3275) (50/box)
Cervical: (Cat. No. 3300) (50/box)
GEN-PROBE® Detection Reagent Kit (Cat. No. 1971) (1200 tests)
GEN-PROBE® LEADER® Luminometer
GEN-PROBE® Magnetic Separation Unit (Cat. No. 1639 or equivalent)
GEN-PROBE® FAST Express Reagent Kit (Cat. No. 2930)
Dispenser, Adjustable Volume (Cat. No. 1714)
Pipettor, Eppendorf Repeating (Cat. No. 2113)
Wash Bottle Cap Assembly (Cat. No. 3919)
GEN-PROBE® Bottle Top Dispenser Adapter Kit (Cat. No. 4173)
GEN-PROBE® Dispenser, 5 mL, Cat. No. 3078

TEST PROCEDURE

A. SAMPLE PREPARATION

1. Allow the specimens to reach room temperature prior to processing.
2. Vortex each GEN-PROBE transport tube for at least 5 seconds.
3. Express all liquid from the swab by pressing the swab against the wall of the tube. Discard the swab.
4. Prior to testing, vortex the transport tube for at least 5 seconds to ensure homogeneity.

B. REAGENT PREPARATION

1. All reagents EXCEPT the Probe Reagent, PACE 2 Hybridization Buffer and Separation Reagent must reach room temperature prior to using. Probe Reagent and Separation Reagent must be maintained at 2° to 8°C until used.
2. Probe Reagent

   Lyophilized Probe
   If the PACE 2 Hybridization Buffer has formed a gel or has been stored at 2° to 8°C, promptly vortex for 10 seconds upon removal. After vortexing, warm the reagent by swirling the vial in a water bath at 60° ± 1°C for 3 to 4 minutes. Vortex again for 10 seconds to ensure a homogeneous solution. It may be necessary to repeat this procedure if the PACE 2 Hybridization Buffer is not homogeneous. Pipette 6.0 mL of PACE 2 Hybridization Buffer into lyophilized PACE 2C *Chlamydia trachomatis*/ *Neisseria gonorrhoeae* Probe Reagent (PACE 2C Probe Reagent). Allow the reagent to stand at room temperature for 2 minutes and then vortex for 10 seconds prior to use. Visually inspect to ensure that the reagent is completely rehydrated and homogeneous. Record on the label the date reconstituted.

   Reconstituted Probe
   The reconstituted PACE 2C Probe Reagent is stable for 3 weeks when stored at 2° to 8°C or until the date stamped on the reagent container, whichever comes first. If the reconstituted PACE 2C Probe Reagent has been refrigerated, vortex for 10 seconds then warm it by swirling the vial in a water bath at 60° ± 1°C for 2 minutes. Prior to use, vortex again for 10 seconds to ensure homogeneity. It may be necessary to repeat this procedure if the reconstituted PACE 2C Probe Reagent is not homogeneous.
3. Separation Suspension

   Determine the number of tests to be performed. Calculate the volumes of Selection Reagent and Separation Reagent as follows:

   Volume of Selection Reagent (mL) = number of tests + 2 extra tests (with Eppendorf repeating pipettor)

   = number of tests + 10 extra tests (with bottle top dispenser)

   Volume of Separation Reagent (mL) = $\frac{\text{Volume of Selection Reagent (mL)}}{20}$

   Pour the required volume of Selection Reagent into a clean dry container. Mix the Separation Reagent, add the required volume to the Selection Reagent, and mix well. Prepared Separation Suspension is stored at room temperature and is stable for 6 hours.

   Separation Suspension Preparation
   (Example)

   8 tests + 2 = 10 tests

Number of Tests	Selection Reagent	Separation Reagent
8 + 2	10 mL	0.5 mL
18 + 2	20 mL	1.0 mL
48 + 2	50 mL	2.5 mL
98 + 2	100 mL	5.0 mL

C. HYBRIDIZATION

1. Label tubes with sample identification numbers. Include three tubes for the Negative Reference, one for the *C. trachomatis* Positive Control, and one for the *N. gonorrhoeae* Positive Control. Label near the tops of the tubes only. The reference and controls must be run with each batch of specimens.
2. Insert the tubes into the tube rack portion of the GEN-PROBE Magnetic Separation Unit. Set aside the base portion of the separation unit for later use.
3. Vortex each specimen for 5 seconds.
4. Pipette 100 µL of each of the controls and specimens to the bottom of the respective tubes.
5. Pipette 100 µL of the PACE 2C Probe Reagent to the BOTTOM of each tube, taking care not to touch the top or sides of the tube.
6. Cover the tubes with Sealing Cards ensuring that each tube is sealed.
7. Shake the rack 3 to 5 times to mix.
8. Incubate the tubes in a water bath at 60° ± 1°C for 1 hour. **DO NOT** place the magnetic separation unit base in the water bath.

D. EQUIPMENT PREPARATION

1. Prepare the GEN-PROBE LEADER for operation. Make sure there is sufficient volume of Detection Reagents I and II to complete the tests.

E. SEPARATION

1. Remove the tube rack from the water bath and remove the Sealing Cards.
2. Pipette 1 mL of the well-mixed, prepared Separation Suspension into each tube.
3. Cover the tubes with Sealing Cards and vigorously shake the tube rack 3 to 5 times to mix. A foam head should be present in each tube.
4. Immediately place the tube rack in a water bath at 60° ± 1°C and incubate for 10 minutes.
5. Remove the tube rack from the water bath. Remove the Sealing Cards and place the tube rack on the base of the Magnetic Separation Unit for 5 minutes at room temperature.
6. Holding the tube rack and base of the GEN-PROBE Magnetic Separation Unit together, decant the supernatants. Before turning tubes upright, shake the unit 2 to 3 times and then blot tubes 3 times for 5 seconds each on absorbent paper.
7. DO NOT REMOVE THE TUBE RACK FROM THE GEN-PROBE MAGNETIC SEPARATION BASE. Fill each tube to the rim with Wash Solution. See PROCEDURAL NOTES regarding Wash Solution addition.

8. Allow the tubes to remain on the magnetic separation base for 20 minutes at room temperature.
9. Holding the tube rack and base together, decant supernatants. Before turning tubes upright, shake the unit 2 to 3 times. DO NOT BLOT. Approximately 50-100 µL of Wash Solution should remain in each tube.
10. Separate the tube rack from the base and shake the tube rack to resuspend the pellets.

F. DETECTION

1. Select the appropriate protocol from the LEADER luminometer software.
2. Using a damp tissue or damp paper towel, wipe each tube to ensure that no residue is present on the outside of the tube. Ensure that the pellets are resuspended and insert the tubes in the LEADER luminometer according to the prompts provided by the instrument software.

   Read the tubes in the following order:

   Negative Reference, 3 tubes
   C. trachomatis Positive Control, 1 tube
   N. gonorrhoeae Positive Control, 1 tube
   Specimen tubes
3. When the analysis is complete, remove the tube(s) from the LEADER luminometer.

PROCEDURAL NOTES

A. PACE 2 HYBRIDIZATION BUFFER: Gel formation of the PACE 2 Hybridiation Buffer and reconstituted Probe Reagent may occasionally occur. Vortexing, heating and swirling of reagents at 60° ± 1°C is imperative to minimize gel formation and ensure a homogeneous solution.

B. SPECIMENS: Occasionally a specimen may be too viscous to pipet. Be sure that specimens are at room temperature and vortex to liquify. The GEN-PROBE FAST Express reagent may be used to simplify specimen preparation. For information, call Gen-Probe Technical Service.

C. PIPETTING: For convenience, repeating pipettors or dispensers may be used for addition of PACE 2C Probe Reagent, Separation Suspension, and Wash Solution. Pipettors with disposable tips are recommended for pipetting specimens, references, and controls to avoid sample carry-over and cross-contamination. Care should be taken to pipette PACE 2C Probe Reagent to the BOTTOM of tubes without inserting the pipette tip into the tubes or touching the tip to the rim of each tube. When adding the reagents, angle the solutions toward the front sides of the tubes, not straight to the bottoms, to avoid splashback.

D. BLOTTING: Discard absorbent paper after each blotting to avoid contamination. DO NOT BLOT AFTER THE WASH STEP.

E. TEMPERATURE: The hybridization and separation reactions are temperature dependent. Therefore, it is imperative that the water bath and reaction tubes be equilibrated uniformly during these steps. A covered water bath capable of maintaining 60° ± 1°C should be used.

F. WASHING: **Forceful addition of the Wash Solution is required.** The Wash Solution should be forcefully injected into each tube. Angle the Wash Solution towards the front sides of the tubes, not straight to the bottoms, to avoid splashback. Appearance of a 1 cm foam head on the reaction tubes will signal forceful enough addition of Wash Solution. After adding Wash Solution to all tubes in the rack, care should be taken to go back and "top off" each tube so no foam remains. Failure to deliver Wash Solution in the specified manner may result in spurious results.

G. WASH BOTTLE AND CAP ASSEMBLY: This is an optional method for delivering Wash Solution. Each laboratory should validate that this assembly yields assay performance equivalent to that of the current method of Wash Solution addition. Prior to using a new wash bottle and cap assembly, pour Wash Solution into the bottle. Screw cap onto bottle. Discard the first 5 mL by squirting through the cap.

H. As in any reagent system, excess powder on some gloves may cause contamination of opened reagents or reaction tubes. Gen-Probe recommends that customers experiencing difficulty with the test avoid using this type of laboratory glove. Using powderless gloves (no talcum powder) will avoid this difficulty.

I. DETECTION: Tubes should be read in the LEADER luminometer within 60 minutes of decanting the Wash Solution. Tubes should be maintained at 20° to 25°C prior to reading.

RESULTS

A. CALCULATION OF RESULTS

The results of the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE are calculated based on the difference between the response in Relative Light Units (RLU) of the specimen and the mean of the Negative Reference.

Mean of the Negative Reference = Sum of the three Negative Reference replicates divided by 3.

Example:

Mean of the Negative Reference =

$$\frac{(65 \text{ RLU} + 71 \text{ RLU} + 80 \text{ RLU})}{3} = 72 \text{ RLU}$$

Assigned Cut-off = 300 RLU

Calculated Cut-off = 300 RLU + 72 RLU = 372 RLU

Specimen Response = 900 RLU Positive

The luminometer prints the specimen response and compares this response to the calculated assay cut-off. A positive or negative interpretation as compared to this cut-off is printed. See the Operator's Manual for detailed protocols.

B. INTERPRETATION OF RESULTS

Results from the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

POSITIVE - The difference is greater than or equal to 300 RLU.

NEGATIVE - The difference is less than 300 RLU.

A positive PACE 2C screening result may indicate the presence of *Chlamydia trachomatis* rRNA and/or *Neisseria gonorrhoeae* rRNA in the specimen tested. Follow-up testing in individual *C. trachomatis* and *N. gonorrhoeae* assays is needed to identify the organism(s) (4).

A negative result should be reported that *Chlamydia trachomatis* rRNA and *Neisseria gonorrhoeae* rRNA were not detected in the specimen tested.

C. QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

NOTE: The Negative Reference and Positive Control provided, control the PACE 2C assay only. They do not control for the lysis of the target organism(s) in the specimen transport medium.

Negative Reference:

The Negative Reference provides a measure of the assay background and is used to calculate the run cut-off. The expected values of the Negative Reference were validated using 69 runs (3 replicates/run) at five locations throughout the United States.

The response of each Negative Reference should be ≤ 200 RLU. All Negative Reference values should fall within 30% of the mean response for the Negative Reference (i.e., the Coefficient of Variance

should be ≤ 30%). If one value falls outside these ranges, it may be deleted from the calculations by following the instructions in the LEADER luminometer Operator's Manual. If two values fall outside these ranges, the test should be repeated. If this is a frequent occurrence, re-evaluate the technique used and call Gen-Probe Technical Service if the problem persists.

Positive Controls:

The expected values for each of the Positive Controls were validated using 69 different runs at five locations throughout the United States.

The difference in the response of each of the Positive Controls and the mean response of the Negative Reference should be > 600 RLU and < 3,200 RLU or the run is invalid. If the Positive Control values repeatedly fall out of specification, contact Gen-Probe Technical Service. Results from the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

If the Positive Controls or Negative Reference values are not in the required ranges, the test results are invalid and must not be reported.

Each laboratory under its normal operating conditions should establish its own mean and range for the Negative Reference and Positive Controls and maintain records according to Standard Laboratory Quality Control practices (2, 21).

Sample Processing Cell Controls:

To test the effectiveness of sample processing, positive and negative cell controls may be run in conjunction with the specimens. For example, *C. trachomatis* ATCC VR878 and *N. gonorrhoeae* ATCC 19424 may be used as the positive cell controls and N. mucosa ATCC 19696 may be used as the negative cell control. For the Neisseria isolates, use actively growing cultures to prepare suspensions of approximately 3 x 10^8 CFU/mL in sterile saline. Inoculate 10 µL of each cell suspension into a transport tube from a GEN-PROBE PACE Specimen Collection Kit (endocervical or urethral/conjunctival) and vortex. For the Chlamydia isolates, use stocks harvested from cell culture and titered to be approximately 3 x 10^6 IFU/mL. Transfer 10 µL of each stock to a transport tube from a GEN-PROBE PACE Specimen Collection Kit (endocervical or urethral/conjunctival) and vortex. Process the controls in the same manner as the patient specimens, beginning with Step C1 of the test procedure. The positive cell controls should produce positive test results and the negative cell controls should produce negative test results.

LIMITATIONS

This method has been tested using endocervical and male urethral swab specimens only. Performance with other specimens has not been assessed.

During routine analysis, bloody specimens have not proven to interfere with assay performance. However, grossly bloody specimens (greater than 80 µL whole blood in 1 mL transport media) may interfere with performance.

The PACE 2C assay has been evaluated for interference by gynecological lubricants and spermicides. The data indicate that in normal usage no interference will be observed. For additional information on particular products, call Gen-Probe Technical Service.

Other endogenous substances that may be present in patient samples may interfere with the assay.

All *Chlamydia trachomatis* and *Neisseria gonorrhoeae* identification methods can yield false positive results. In those circumstances where diagnosis could lead to adverse psychosocial impacts, additional testing methods are recommended. Culture is the only recommended procedure for diagnosing chlamydial and gonorrheal infection in medicolegal cases.

As in any disease state, the positive predictive value of this assay will decrease as the prevalence decreases in the population. Reliable results are dependent on adequate specimen collection. Because the transport system used for this assay does not permit microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary. See the Sample Collection and Storage section of this insert for instructions.

Therapeutic failure or success cannot be determined as nucleic acid may persist following appropriate antimicrobial therapy.

Results from the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

A negative test does not exclude the possibility that the numbers of *C. trachomatis* and/or *N. gonorrhoeae* organisms may be below the level of detection of the assay. A second swab can be collected and cultured to identify those patients infected with low levels of organism(s). As well, test results may be affected by improper specimen collection, technical error, specimen mix-up or concurrent antibiotic therapy.

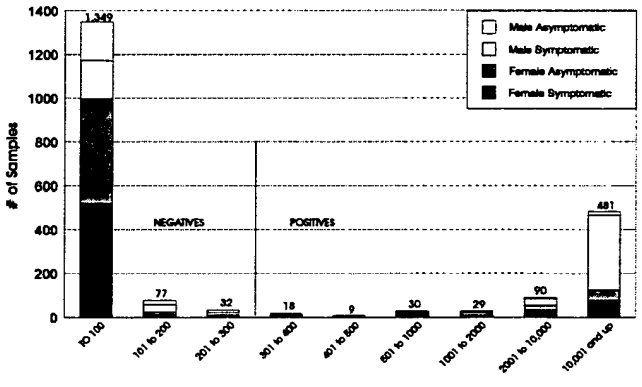
If a positive PACE 2C result contradicts other clinical or patient information or if the patient belongs to a category cited in the 1993 CDC *C. trachomatis* guidelines (CDC, MMWR, Vol. 42, No. RR-12, 1993), verification of the result may be warranted.

Because the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE is a screening assay designed to detect the presence of *C. trachomatis* and/*or N. gonorrhoeae*, and because of the possibility of dual infection, follow-up testing of PACE 2C-positives in individual *C. trachomatis* and *N. gonorrhoeae* assays is needed.

EXPECTED VALUES

DISTRIBUTION OF CLINICAL RESULTS

Using the results obtained for the specimens tested in the clinical trial for PACE 2C, a distribution of sample RLU values above and below the assay cut-off was generated. The data are presented below after resolution of discrepant specimens. The numbers of false positive (FP) and false negative (FN) results for each RLU category are given below the figure.



FP			13	2	14	11	8	7
FN	10	10	11					

PERFORMANCE CHARACTERISTICS

A. WITHIN-RUN PRECISION

The within-run precision of the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE was calculated by assaying four concentrations of *C. trachomatis* inclusion forming units (IFU) and *N. gonorrhoeae* cells using five replicates in a single assay; one negative sample was also run.

Sample	A	B	C	D	E
Number of Replicates	5	5	5	5	5
Mean Response (RLU)	9530	2075	1041	767	49
Standard Deviation (RLU)	210	143	42	52	2
Coefficient of Variance	2.2%	6.9%	4.0%	6.8%	n/a

B. BETWEEN-RUN PRECISION

Between-run precision was calculated by assaying the same four concentrations of *C. trachomatis* IFU and *N. gonorrhoeae* cells and one negative sample using the average of five replicates determined in three consecutive runs.

Sample	A	B	C	D	E
Number of Replicates	3	3	3	3	3
Mean Response (RLU)	10147	2259	968	690	51
Standard Deviation (RLU)	917	240	66	74	4
Coefficient of Variance	9.0%	10.6%	6.8%	10.7%	n/a

C. POSITIVE CONTROL PRECISION

Precision data for the *C. trachomatis* and *N. gonorrhoeae* Positive Controls were determined in PACE 2C assays performed at five locations throughout the United States. One replicate of each Positive Control was assayed in each PACE 2C run.

Sample	*C. trachomatis* Positive Control	*N. gonorrhoeae* Positive Control
Number of Replicates	69	69
Mean Response (RLU)	1837	2165
Standard Deviation (RLU)	329	425
Coefficient of Variance	5.6%	5.1%

D. ANALYTICAL SENSITIVITY

The analytical sensitivity (limits of detection) of the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE was determined by directly comparing dilutions of freshly grown *C. trachomatis* and *N. gonorrhoeae* in cell culture and in the PACE 2C assay. The sensitivities for the 15 *C. trachomatis* serovars at the assay cut-off of 300 RLU plus the mean of the Negative Reference ranged from 24-2,232 inclusion-forming units (IFU)/assay (0.1 mL inoculated transport medium); the average was 966 IFU/assay. The sensitivity of *N. gonorrhoeae* was determined to be approximately 650 colony-forming units (CFU)/assay (0.1 mL inoculated transport medium).

E. ANALYTICAL SPECIFICITY

A total of 80 culture isolates were evaluated using the PACE 2C assay. These isolates included 20 organisms that may be isolated from the urogenital tract and 30 additional organisms that represent a phylogenetic cross-section of organisms. Culture isolates of *C. trachomatis* (15 serovars), *N. gonorrhoeae*, *Chlamydia psittaci*, *Chlamydia pneumoniae*, and 12 species of *Neisseriaceae* were also tested. Only the *C. trachomatis* and *N. gonorrhoeae* samples produced a positive result in the GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE.

F. RECOVERY

Ribosomal RNA isolated from *C. psittaci, Ureaplasma urealyticum,* and *Neisseria meningitidis* was added at a concentration of 0.1 µg/assay to samples containing different concentrations of *C. trachomatis* and/or *N. gonorrhoeae* ribosomal RNA. These additions did not interfere with the recovery of *C. trachomatis* or *N. gonorrhoeae* rRNA using the PACE 2C assay.

G. CLINICAL TRIAL RESULTS

The GEN-PROBE PACE 2C System for CHLAMYDIA TRACHOMATIS and NEISSERIA GONORRHOEAE was compared to standard culture methods for *C. trachomatis* and *N. gonorrhoeae* using 1,266 endocervical specimens and 849 male urethral specimens. Specimens were evaluated at a total of five clinical sites using a 300 net RLU cut-off. Clinical data are presented below, both before and after resolution of discrepant specimens.

1. PERFORMANCE SUMMARY: BEFORE DISCREPANT RESOLUTION

PACE 2C Culture	Pos Pos	Pos Neg	Neg Pos	Neg Neg	Sensitivity / Specificity (%)
Population (%CT/%NG prevalence; # sites)					
Female					
Symptomatic					
High Prevalence (13.6% / 16.2%; 2 sites)	74	9	6	219	92.5/96.1
Low prevalence (8.9% / 7.0%; 2 sites)	48	8	3	313	94.1/97.5
Female					
Asymptomatic					
High Prevalence (12.1% / 17.0%; 2 sites)	71	6	8	221	89.9/97.4
Low Prevalence (3.2% / 1.8%; 2 sites)	12	4	1	263	92.3/98.5
Male					
Symptomatic (11.5% / 55.7%; 4 sites)	371	17	11	208	97.1/92.4
Asymptomatic (6.6% / 5.4%; 4 sites)	26	11	2	203	92.9/94.9
Combined (10.1% / 41.3%; 4 sites)	397	28	13	411	96.8/93.6

2. PERFORMANCE SUMMARY: AFTER DISCREPANT RESOLUTION

Discrepant samples for *C. trachomatis* were resolved by re-culture and DFA. Cell culture was not repeated for *N. gonorrhoeae* discrepant samples.

PACE 2C Culture Population	Pos Pos	Pos Neg	Neg Pos	Neg Neg	Sensitivity/ Specificity (%)	95% Confidence Intervals Sensitivity/ Specificity (%)
Female Symptomatic						
High Prevalence	77	6	6	219	92.8/97.3	86.7-97.9 / 94.7-99.1
Low Prevalence	49	7	3	313	94.2 / 97.8	86.5-100.0 / 95.6-99.1
Female Asymptomatic						
High Prevalence	71	6	8	221	89.9 / 97.4	81.0-94.9 / 94.7-99.1
Low Prevalence	13	3	1	263	92.9 / 98.9	71.4-100.0 / 97.4-100.0
Male						
Symptomatic	373	15	11	208	97.1 / 93.3	95.3-98.7 / 89.7-96.0
Asymptomatic	28	9	2	203	93.3 / 95.8	83.3-100.0 / 92.5-98.1
Combined	401	24	13	411	96.9 / 94.5	94.9-98.3 / 92.0-96.3

Of the 55 total PACE 2C-positive, culture-negative samples, 15 were negative when tested in the individual PACE 2 assays for *C. trachomatis* and *N. gonorrhoeae*. As well,

although it could not be included in the discrepant resolution protocol described above, a research amplification assay was used to test a number of the apparent PACE 2C false positives. Of 40 PACE 2C-positive, culture-negative probe samples tested in amplification, 26 demonstrated the presence of *C. trachomatis* nucleic acid and 11 *N. gonorrhoeae* nucleic acid. These data indicate that a majority of samples classified in the above table as PACE 2C false positives were, in fact, true positives that were missed by cell culture. The samples remained classified as false positives because the amplification assay used was a research assay.

3. PERFORMANCE SUMMARY: INDIVIDUAL ORGANISMS

The ability of PACE 2C to detect *C. trachomatis* and *N. gonorrhoeae* individually was determined by analyzing the resolved PACE 2C vs. culture results of positive samples separately for each of the two target organisms. In order to simplify the analysis, dual positive samples were included in both the *C. trachomatis* and *N. gonorrhoeae* data. Therefore, the total number of positive samples in the table below is increased by 59 over the number in the other clinical data tables.

	PACE 2C	
	Positive	**Negative**
C. trachomatis Culture Positive		
Female	109	12
Male	79	12
N. gonorrhoeae Culture Positive		
Female	132	6
Male	350	1

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